

# Waters

## AMINO ACID ANALYSIS FOR MONITORING PROTEIN STRUCTURE AND FOR MEASURING PROTEIN CONCENTRATION

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### OVERVIEW

A new total application solution for the analysis of amino acids has been recently developed. The analysis provides better resolution and sensitivity than has been possible in existing methods. The enhanced separation ensures that the analysis yields accurate and precise qualitative and quantitative results and that the method is rugged. The method, based on the well understood and widely used AccQ•Tag™ pre-column derivatization chemistry, provides these benefits in a shorter analysis time than previously possible. The derivatives are separated using Waters® ACQUITY UltraPerformance LC® (UPLC®) for optimum resolution and sensitivity. System control, data acquisition, processing, and flexible reporting are provided within Empower™ software. The integrated total application solution ensures successful analyses.

### UPLC® AMINO ACID ANALYSIS SOLUTION

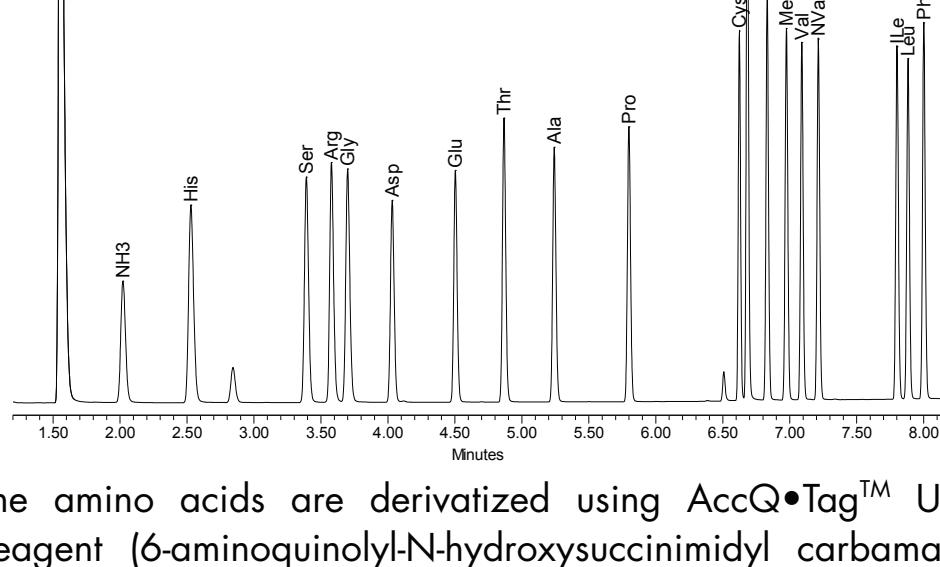
#### Design Considerations and Criteria for Success

- Complete resolution of all amino acids
- Adequate resolution for unambiguous identification
- Complete resolution of derivatization by-products from amino acid derivatives
- 3 orders of magnitude linear dynamic range for quantitation
- Adequate electrospray ionization sensitivity and stability

#### Complete Turn-key AAA System

- ACQUITY UPLC system provides enhanced resolution for better accuracy, robustness, sensitivity, and speed
- AccQ•Tag™ Ultra Column and packaged reagents/elutents specifically QC tested with amino acid analysis

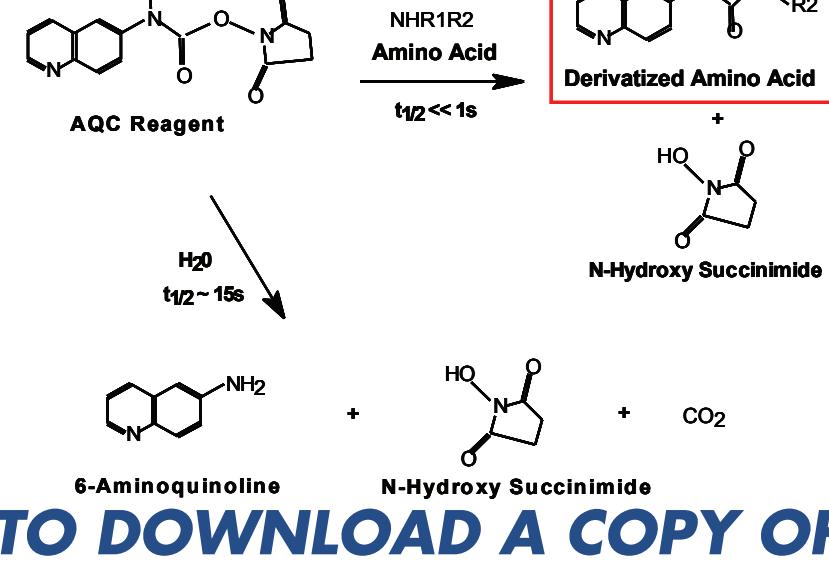
### STANDARD ANALYSIS



The amino acids are derivatized using AccQ•Tag™ Ultra Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). Both primary and secondary amino acids react in a simple batch-wise derivatization.

- Reaction occurs in largely aqueous solution and is therefore tolerant of buffer salts and sample components
- No special sample handling, vacuum drying or extraction is required
- Samples are stable for several days
- Excess reagent naturally hydrolyzes
- Reagent by-product is chromatographically resolved from derivatives
- The derivatives are separated with a UPLC method using a QC-tested column and elutents

### DERIVATIZATION CHEMISTRY



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### REPRODUCIBILITY

	Mean	Std Dev	%RSD
AMQ	1.459	0.017	1.19
NH3	1.915	0.024	1.26
His	2.401	0.032	1.34
Ser	2.250	0.030	0.93
Arg	2.463	0.027	0.79
Gly	3.556	0.028	0.78
Asp	3.897	0.028	0.72
Glu	4.387	0.024	0.54
Thr	4.766	0.021	0.43
Ala	5.145	0.019	0.37
Pro	5.710	0.016	0.28
Cys	6.569	0.011	0.17
Lys	6.638	0.011	0.16
Tyr	6.768	0.014	0.21
Met	6.917	0.013	0.19
Val	7.040	0.012	0.17
Nva	7.165	0.012	0.17
Ile	7.761	0.011	0.14
Leu	7.844	0.011	0.14
Phe	7.957	0.011	0.14

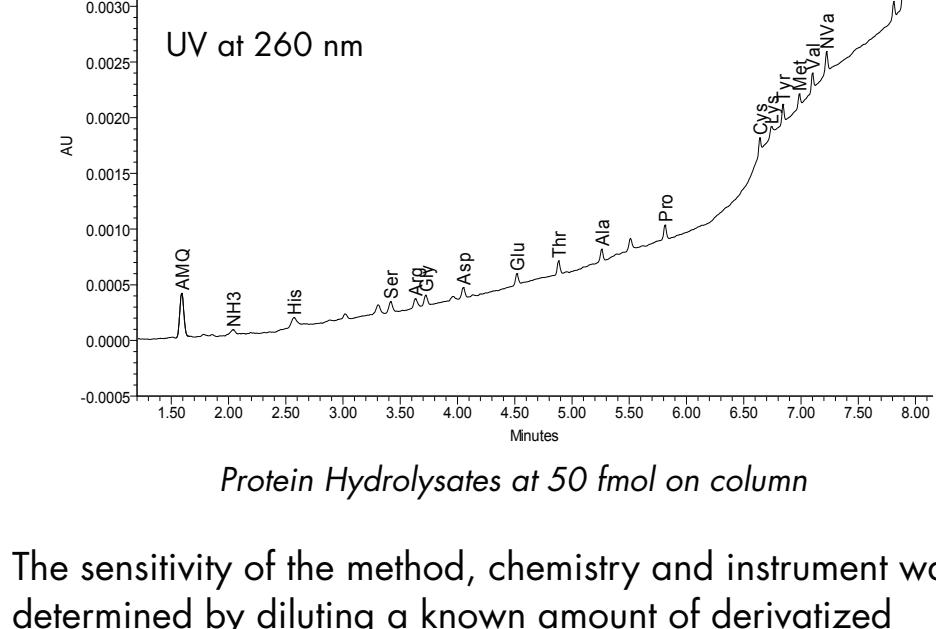
Most Variable

Most Closely Spaced

Retention Time Reproducibility of 25 AccQ•Tag Ultra Columns

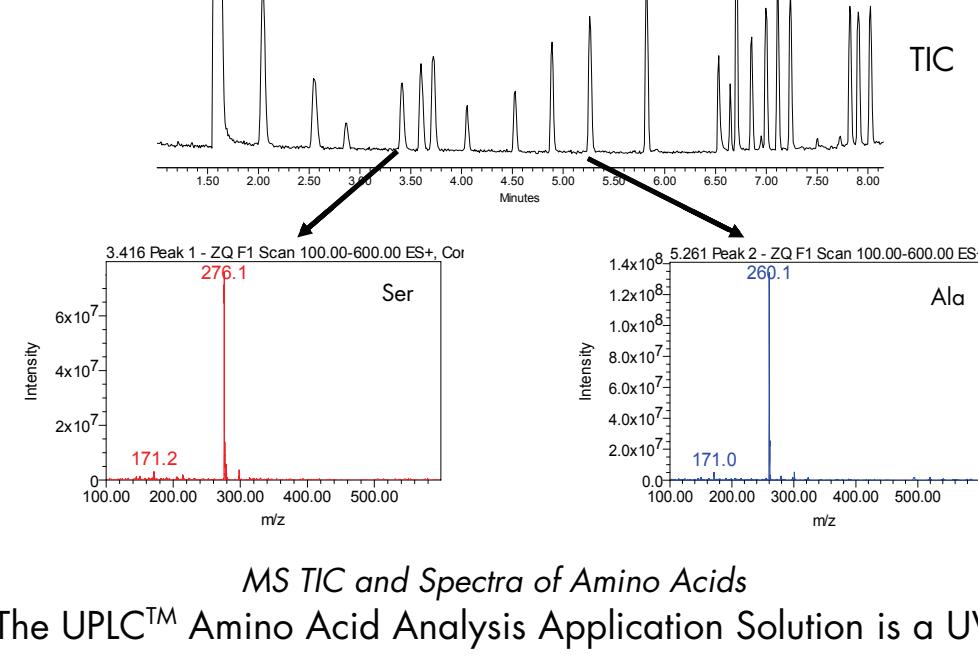
Peaks in amino acid analysis are identified by retention time. Therefore, qualitative analysis depends upon both resolution and chromatographic reproducibility. The retention time reproducibility of 25 AccQ•Tag Ultra columns was tested by 2 operators on 4 systems. Standard deviations range from 0.011 min (0.7 sec) to 0.032 min (2 sec). To determine whether misidentification can occur, we can focus on the most variable peak (Histidine) and the most closely spaced pair (Cys/Lys). A retention time window that is +/- 2 std dev around the mean can be defined for each peak. The window for Histidine does not overlap the windows for the adjacent peaks. Similarly, the windows for the closely spaced Cys and Lys peaks do not overlap. The combination of good resolution and reproducibility ensures that there will be no ambiguity in peak identification.

### METHOD SENSITIVITY



The sensitivity of the method, chemistry and instrument was determined by diluting a known amount of derivatized sample. Levels as low as 50 femtmoles on column were achieved. Sensitivity for samples will be limited by environmental background and contamination.

### ESI-MS COMPATIBILITY

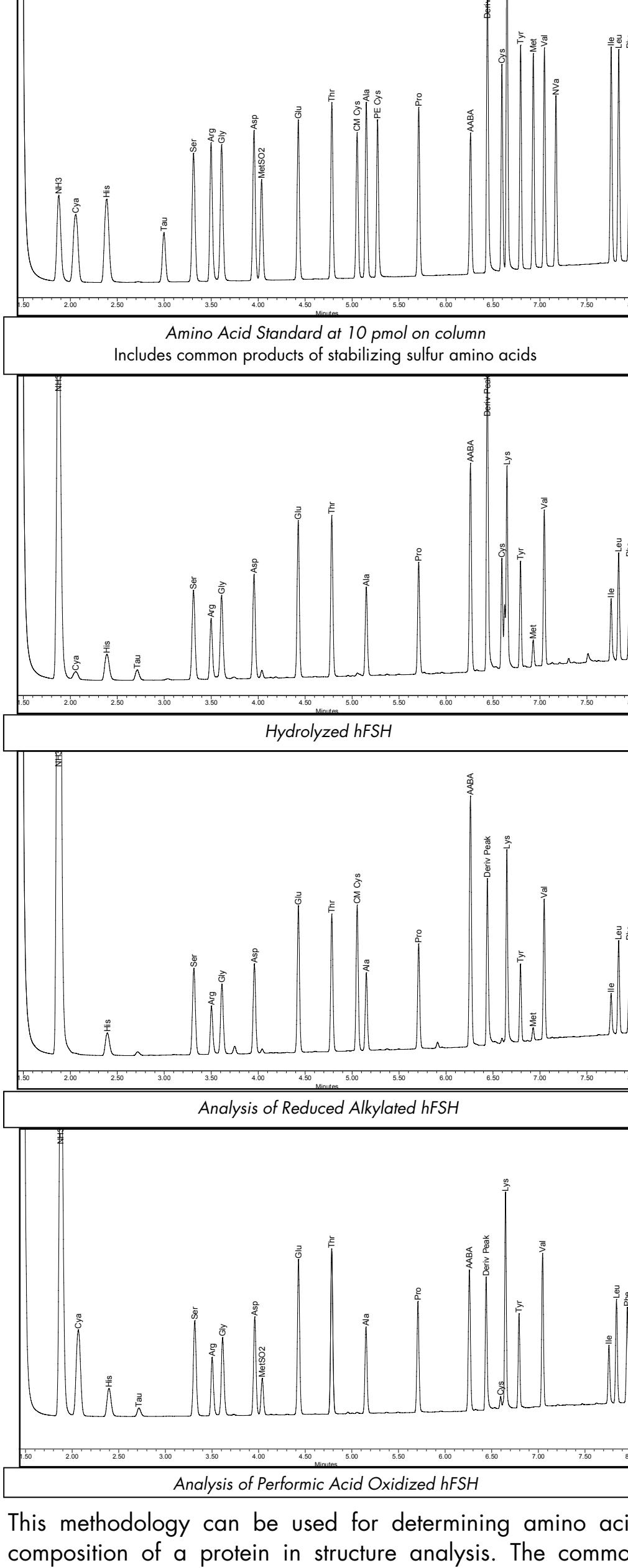


MS TIC and Spectra of Amino Acids

The UPLC™ Amino Acid Analysis Application Solution is a UV based method that uses mobile phase elutents that are directly compatible with electrospray mass spectrometry. MS detection is not required for routine peak identification and is not necessary to detect sub picomole levels. However, it is a valuable tool in regards to the following determinations:

- Confirmation of amino acids by molecular weight
- Valuable for initial validation of a method
- Useful in determining unknown or unexpected peaks in a sample

### PROTEIN ANALYSIS



This methodology can be used for determining amino acid composition of a protein in structure analysis. The common products of stabilizing sulfur-containing amino acids can be measured without modification of the standard method.

The analysis can be calibrated as the residue weight of each amino acid. The sum of amino acid weights per injection equals the mass of protein injected. The amount actually submitted for hydrolysis and the concentration of the original sample can then be calculated from the known dilution factors.

Sample	hFSH Hydrolysate	hFSH Reduced Carboxymethylation/Hydrolysate	hFSH Performic Acid Oxidation/Hydrolysate
Measured amount of protein in tube (μg)	3.43	2.71	5.69

### CONCLUSIONS

- The UPLC Amino Acid Analysis Solution provides a robust, turnkey AAA method.
- The standard method gives accurate and precise results in a very short run time.
- The method can be used with electrospray ionization mass spectrometry without modification.
- The results are suitable for use in structural analysis, including the analysis of sulfur-containing amino acids.

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